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High-density lipoproteins and endothelial functions: Mechanistic insights and alterations in cardiovascular disease

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Abstract: Prospective studies of cardiovascular risk-factors have shown that reduced plasma levels of HDL-cholesterol are associated with increased risk of coronary-disease. Experimental and translational studies have revealed several potential anti-atherogenic effects of HDL, including protective effects of HDL on endothelial cell functions. HDL has been suggested to protect endothelial cell functions by preventing oxidation of LDL and its adverse endothelial effects. Moreover, HDL from healthy subjects can directly stimulate endothelial cell production of nitric-oxide, anti-inflammatory, anti-apoptotic, anti-thrombotic effects, and endothelial-repair processes. Recent clinical-trials using HDL-cholesterol raising agents, such as torcetrapib, dalcetrapib and niacin, could not observe significant reduction of cardiovascular events in patients with coronary-disease. Growing evidence suggests that vascular effects of HDL are highly heterogenous and vasoprotective properties of HDL are altered in patients with coronary-disease. Notably, in recent studies no clear association of higher HDL-cholesterol levels with reduced risk of cardiovascular events was observed in patients with established coronary-disease. Greater understanding of mechanisms of action of HDL and its altered vascular effects is therefore crucial within the context of HDL-targeted therapies. In this review, we will address different effects of HDL on endothelial cell functions potentially relevant to atherosclerotic vascular disease and explore molecular mechanisms leading to dysfunctional HDL.

DOI: <https://doi.org/10.1194/jlr.R037762>

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ZORA URL: <https://doi.org/10.5167/uzh-83897>

Journal Article

Accepted Version

Originally published at:

Riwanto, Meliana; Landmesser, Ulf (2013). High-density lipoproteins and endothelial functions: Mechanistic insights and alterations in cardiovascular disease. *Journal of Lipid Research*, 54(12):3227-3243.

DOI: <https://doi.org/10.1194/jlr.R037762>

**High-density lipoproteins and Endothelial Functions:
Mechanistic Insights and Alterations in Cardiovascular Disease**

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Total word count: 7958

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Subject codes: High density lipoprotein; Endothelium; Atherosclerosis; Coronary disease

Abstract

Prospective population studies in the primary prevention setting have shown that reduced plasma levels of HDL cholesterol are associated with an increased risk of coronary disease and myocardial infarction. Experimental and translational studies have further revealed several potential anti-atherogenic effects of HDL, including protective effects on endothelial cell functions. HDL has been suggested to protect endothelial cell functions by prevention of oxidation of LDL and its adverse endothelial effects. Moreover, HDL from healthy subjects can directly stimulate endothelial cell production of nitric oxide, anti-inflammatory, anti-apoptotic and anti-thrombotic effects as well as endothelial repair processes.

However, several recent clinical trials using HDL cholesterol raising agents, such as torcetrapib, dalcetrapib and niacin, did not demonstrate a significant reduction of cardiovascular events in patients with coronary disease. Of note, growing evidence suggests that the vascular effects of HDL can be highly heterogeneous and vasoprotective properties of HDL are altered in patients with coronary disease. Characterization of underlying mechanisms and understanding of the clinical relevance of this “HDL dysfunction” are currently an active field of cardiovascular research. Notably, in some recent studies no clear association of higher HDL cholesterol levels with a reduced risk of cardiovascular events was observed in patients with already established coronary disease. A greater understanding of mechanisms of action of HDL and its altered vascular effects is therefore critical within the context of HDL-targeted therapies. In this review, we will address different effects of HDL on endothelial cell functions potentially relevant to atherosclerotic vascular disease and explore molecular mechanisms leading to “dysfunctional HDL”.

Introduction

Large prospective studies of cardiovascular risk factors have shown that reduced plasma levels of HDL cholesterol are associated with an increased risk of coronary artery disease (CAD) (1-5). In recent years, several biological functions of HDL have been identified, whereby HDL may exert anti-atherogenic effects (6-8); e.g. HDL from healthy subjects has been shown to directly promote endothelial anti-inflammatory, anti-apoptotic and anti-thrombotic effects (7, 9-12) (Figure 1). Accordingly, interventions to increase HDL-cholesterol levels and/or HDL-function are being intensely evaluated as a potential therapeutic strategy to reduce cardiovascular risk. However, recent evidence suggests that the endothelial and vascular effects of HDL are highly heterogeneous and vasoprotective properties of HDL are impaired in patients with diabetes, coronary disease or chronic kidney dysfunction (13-16) (Figure 1).

Various studies have examined the association of genetic variations leading to altered HDL cholesterol plasma levels with coronary disease risk (17-19). These studies suggest that some genetic variations associated with higher HDL cholesterol plasma levels may not necessarily be associated with a reduced risk of coronary disease or myocardial infarction, although this does not apply for all associations (eg. CETP) and for some genes remains inconclusive (20, 21).

Of note, several recent clinical trials have not been able to demonstrate that therapies that increase HDL cholesterol levels reduce cardiovascular risk in patients with coronary disease. The Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic Events (ILLUMINATE) trial testing the impact of the CETP inhibitor torcetrapib on clinical outcome showed an increase in cardiovascular events and total mortality, despite elevations in HDL cholesterol, that was, however, at least in part attributed to toxic effects of the compound (22). Dalcetrapib, another CETP inhibitor, raised the HDL-cholesterol level in patients hospitalized with an acute coronary syndrome, but the trial was terminated before completion due to lack of clinical benefit (23). And very recently, the HPS2-THRIVE trial results showed that adding extended-release niacin/laropiprant, another HDL-raising agent, to statins did not reduce the risk of

cardiovascular events (24). Taken together, these observations strongly suggest that plasma levels of HDL cholesterol alone are not an optimal therapeutic target.

Importantly, accumulating evidence suggests that the vascular effects of HDL can be highly heterogeneous. We and others have observed that HDL loses potential anti-atherosclerotic properties in patients with chronic inflammatory disorders, such as the antiphospholipid syndrome (25), systemic lupus erythematosus and rheumatoid arthritis (26), scleroderma (27), the metabolic syndrome (28), diabetes (13, 29), and coronary disease (15, 16, 30). Notably, in a study of 189 patients with chronic kidney disease on hemodialysis an impaired anti-inflammatory capacity of HDL was correlated with a poor clinical outcome (31). Furthermore, HDL isolated from subjects with type 1 or type 2 diabetes mellitus or abdominal obesity had reduced capacity to reverse the inhibition of aortic ring endothelium-dependent relaxation by oxLDL as compared to HDL from healthy control subjects (29, 32, 33). These proinflammatory HDL particles have been termed 'dysfunctional' HDL (Figure 1). The heterogeneity of the vascular effects of HDL may be attributed to changes in the HDL-associated proteome and lipids, i.e. post-translational protein modifications and changes in the amount and type of proteins and lipids bound to the HDL particle. In particular, high-density lipoprotein is susceptible to oxidation/modification *in vitro* by a variety of oxidants, such as metal ions, peroxy and hydroxyl radicals, aldehydes, various myeloperoxidase (MPO)-generated oxidants, lipoxygenase, phospholipase A2, elastase, non-enzymatic glycation and homocysteinylation (34). In the present review, we will address different mechanisms whereby HDL exert effects on endothelial cell functions. In particular, the effects of HDL on regulation of endothelial nitric oxide synthase (eNOS) and endothelial cell nitric oxide (NO) production, endothelial inflammatory activation, endothelial apoptotic regulation, endothelial repair from vascular injury, lipid oxidation and endothelial thrombotic activation will be discussed. Importantly, recent insights into molecular mechanisms leading to "HDL dysfunction" in different pathophysiological states will be described.

Impact of HDL on LDL oxidation and its endothelial effects: *Lessons from experimental studies and alterations of HDL from patients with coronary disease or diabetes*

HDL may exert endothelial-protective effects by limiting oxidation of LDL. Oxidation of LDL has long been suggested as a relevant mechanism for atherogenesis (35, 36). LDL is entrapped in the subendothelial space where it is subject to oxidative modifications by reactive nitrogen species, myeloperoxidase pathways and others (37). Once formed, oxidized LDL (oxLDL) is a potent inducer of endothelial expression of inflammatory molecules (38). OxLDL also promotes the differentiation of monocytes into macrophages that take-up oxLDL in a process that converts them into foam cells, hallmark cells of atherosclerotic plaques (39).

Hessler et al. early on reported that HDL protects against LDL-induced cytotoxicity on endothelial cells (40). HDL was demonstrated to prevent copper-induced LDL oxidation or LDL oxidation by cultured endothelial cells (41-43). HDL is a major carrier of lipid peroxidation products (44, 45) which are thought to play a role in the initiation and progression of atherosclerotic vascular disease (46). HDL can directly inhibit oxidation of low-density lipoprotein via transfer of oxidation products from LDL to HDL (44). In addition, circulating HDL accumulates oxidized phospholipids, such as hydroperoxides, lysophosphatidylcholine (lyso-PC) and F2-isoprostanes (44, 45). The transfer of lipid hydroperoxides from LDL prevents the initiation of a free radical chain reaction of oxidation (47). Furthermore, some of the advanced products of phospholipid oxidation may serve as ligands for scavenger receptor and promote uptake of modified lipoproteins by macrophages as well as prothrombotic effects mediated by platelet scavenger receptor CD36 (48, 49).

A study by Navab et al. demonstrated that apoA-I binds to and removes lipid hydroperoxides of LDL *in vitro* and *in vivo* (47). Treatment of human artery wall cells with apoA-I, but not apoA-II, or treatment with an apoA-I peptide mimetic, or with normal HDL, or paraoxonase, also rendered the cells unable to oxidize LDL (50).

Human HDL can also directly reduce cholesteryl ester hydroperoxides and phosphatidylcholine hydroperoxides via Met residues 112 and 148 of apoA-I (51). Recombinant HDL containing only apoA-I and 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC) was as effective as native HDL in preventing LDL oxidation, supporting a key anti-oxidant role for apoA-I (52). *In vivo* studies have demonstrated that apoA-I can act as an anti-oxidative, anti-inflammatory and anti-atherosclerotic agent (53-55). Recent clinical studies have suggested that although very high HDL-cholesterol and large-size HDL particles levels may be associated with an increased cardiovascular risk, a high concentration of apoA-I was an independent negative predictor of cardiovascular risk (56).

Interestingly, several other HDL-associated apolipoproteins have also been shown to exert anti-oxidant effects. ApoA-II-enriched HDL from mice transgenic for human apoA-II protected VLDL from oxidation more efficiently than control HDL (57). However, in other studies, overexpression of human apoA-II in dyslipidemic mice accelerated atherosclerosis and reduced anti-oxidative activity of HDL (58, 59). The authors postulated that the proatherogenic actions of apoA-II may be related to the displacement of apoA-I and PON1 by apoA-II from HDL particles (58). Of note, in a nested case-control study in the prospective EPIC-Norfolk (European Prospective Investigation into Cancer and Nutrition-Norfolk) cohort, apoA-II was found to be associated with a decreased risk of future CAD in apparently healthy subjects (60).

Apolipoprotein E has been shown to have allele-specific anti-oxidant activity (61). Apolipoprotein E2 can stimulate endothelial NO release and has anti-inflammatory activities (62). In contrast, apolipoprotein E4 has been described as pro-inflammatory (63). It has also been reported that HDL-associated apolipoprotein J can inhibit LDL oxidation by artery wall cells (64). In addition, apoA-IV has been demonstrated to exert anti-oxidant, anti-inflammatory and anti-atherosclerotic actions *in vivo* (65-67).

Notably, HDL carries also anti-oxidant enzymes that may be involved in prevention of lipid oxidation or degradation of lipid hydroperoxides, such as PON1, LCAT and platelet-activating factor acetylhydrolase (PAF-AH). In particular, PON1 has been suggested to be an important regulator of the potential anti-

atherogenic capacity of HDL (68, 69). Various studies have suggested that the direct anti-oxidant effect of HDL on LDL oxidation, measured as a reduction in lipid peroxides, is to a significant extent mediated by PON1 (70-72). In human studies, higher PON1 activity is associated with a lower incidence of major cardiovascular events and conversely reduced activity of PON1 is associated with pathological conditions such as chronic renal failure, rheumatoid arthritis and Alzheimer's disease, as reviewed elsewhere (73).

Biochemical studies have suggested the anti-oxidant role of LCAT through its capacity to hydrolyze oxidized acyl chains from phosphatidylcholine-based OxPL and oxidized free fatty acids (74, 75). *In vivo* study in mice deficient for LDL-receptor and leptin showed that LCAT overexpression decreased autoantibodies to oxLDL (76). PAF-AH is another HDL-associated enzyme that can hydrolyze oxidized phospholipids (77, 78). In arteries of non-hyperlipidaemic rabbits, local expression of PAF-AH reduced the accumulation of oxidatively modified LDL without changing plasma levels of PAF-AH and reduced the expression of endothelial cell adhesion molecules (79). In human studies, PAF-AH deficiency through a missense mutation of the gene is an independent risk factor for coronary artery disease in Japanese men (80). Circulating levels of PAF-AH is also shown to be an independent marker of the risk of CAD (81). However, in a recent study by Holleboom et al., reduced LCAT activity and PAF-AH activity due to *LCAT* mutations was not associated with increased plasma lipid peroxidation (82).

HDL has also been shown to promote efflux of 7-ketocholesterol (83, 84) at sites of inflammation and thereby reduces endothelial cell inflammatory activation (85). Accordingly, Nicholls et al. have reported that reconstituted HDL inhibits superoxide production and vascular inflammation induced by a non-occlusive carotid periarterial collar in normocholesterolemic rabbits (54). In addition, Van Linthout et al. have observed that human apoA-I gene transfer in rats with streptozotocin-induced diabetes mellitus resulted in a 1.9-fold increase in HDL cholesterol levels and inhibition of angiotensin II type 1 receptor-mediated NAD(P)H oxidase activation and generation of reactive oxygen species (86).

Alterations of the effects of HDL from patients after surgery, with CAD or diabetes on LDL oxidation

Early studies by van Lenten et al. have demonstrated that the anti-inflammatory capacity of HDL is affected by acute phase responses in both humans and rabbits (87). The authors isolated human HDL from the patients before and immediately after surgery and characterized the effects of HDL on LDL-induced monocyte transmigration and lipid hydroperoxide formation (87). Before cardiac surgery, HDL completely inhibited the LDL-induced increase in monocyte transmigration and lipid hydroperoxide formation. In marked contrast, “acute phase” HDL obtained from the same patients 2-3 days after surgery amplified the LDL-induced monocyte transmigration and was less effective in inhibiting lipid hydroperoxide formation, i.e. HDL in the same patient had been transformed from an anti-inflammatory towards a pro-inflammatory particle (87). Interestingly, the changes in HDL functionality in this study were paralleled by an increase in HDL-associated acute phase reactants (i.e. ceruloplasmin and serum amyloid A), while the activities of the HDL-associated anti-oxidant enzymes paraoxonase and platelet-factor activating acetylhydrolase were reduced in acute phase HDL (87). Similarly, an acute Influenza A infection in wild type mice progressively impaired the ability of HDL to inhibit LDL oxidation and LDL-induced monocyte chemotactic activity in human artery wall cell co-cultures up to 9 days after inoculation (88).

HDL from patients with CAD failed to prevent LDL oxidation (50) and HDL from mice genetically predisposed to diet-induced atherosclerosis became proinflammatory when the mice were fed an atherogenic diet (89). A subsequent study by Ansell et al. suggested that the capacity of HDL to alter LDL-induced monocyte chemotactic activity in patients with CAD was somewhat improved after 6 weeks of simvastatin therapy (30). However, HDL from patients with CAD on statin therapy remained proinflammatory in contrast to HDL from age- and sex-matched healthy subjects.

Navab et al. developed a fluorescent cell-free assay to detect the capacity of HDL to inhibit the oxidation of LDL, or inhibit the oxidation of 1- α -1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphorylcholine (PAPC) by hydroperoxyoctadecadienoic acid (HPODE), or inactivate oxidized PAPC (Ox-PAPC) (90).

Using this assay, HDL isolated from 27 patients with coronary atherosclerosis failed to inhibit the fluorescent signal generated by a control LDL, whereas HDL from 31 matched normal subjects with the same levels of HDL cholesterol significantly inhibited the signal (90).

The presence of oxidized lipids in HDL has been proposed to play a role in the altered anti-oxidant properties of HDL (91). Administration of apoA-1 mimetic peptides, L-4F, to apoE deficient mice has been shown to reduce plasma levels of oxidized fatty acids (15-HETE, 5-HETE, 13-HODE and 9-HODE) and improve the HDL anti-oxidant capacity and the capacity of HDL to inhibit LDL-induced monocyte chemotactic activity in cultured human aortic endothelial cells (92). Moreover, in a recent study by Morgantini et al., HDL from patients with type 2 diabetes had impaired anti-oxidant properties and increased oxidized fatty acids content (93). The authors postulated that elevated content of oxidized fatty acids (5-HETE, 9-HETE, 12-HETE, 15-HETE, 9-HODE, and 13-HODE) in HDL isolated from the type 2 diabetics patients may account for the impaired anti-oxidant properties of the lipoprotein (93). In another study, the ability of HDL to inhibit LDL oxidation was found to be reduced in ACS but not in stable CAD patients (94). Very recently, the same group also reported that the anti-oxidative and cholesterol efflux capacities of HDL are reduced in ischaemic cardiomyopathy (95).

Effects of HDL on endothelial NO-Synthase dependent Nitric Oxide production: *Experimental studies and altered effects of HDL from patients with cardiovascular disease*

Endothelial nitric oxide plays a crucial role in the regulation of vascular tone and structure. Endothelial NO synthase derived NO has been shown to exert a variety of athero-protective effects in the vasculature, such as anti-inflammatory and anti-thrombotic effects (96). Reduced endothelial NO bioavailability has therefore been suggested to promote initiation and progression of atherosclerosis (96).

Accumulating evidence suggests that HDL can directly stimulate endothelial NO synthase mediated NO production via endothelial SR-BI (9). Several experimental studies have consistently demonstrated the

capacity of HDL to modulate eNOS expression and to stimulate endothelial NO production *in vitro* and *in vivo* (10, 13, 15, 97-99). Moreover, in human studies, administration of reconstituted HDL has been shown to improve endothelial function in subjects with hypercholesterolemia and in subjects with isolated low HDL due to heterozygous loss-of-function mutations in the ABCA-1 gene locus (100, 101). It is worth noting that one can only speculate on how exactly the reconstituted HDL mixes with endogenous circulating HDL.

Several mechanisms have been proposed to account for the endothelial NO-stimulating capacity of HDL. Early studies have shown that HDL prevents oxLDL-mediated eNOS displacements from caveolae and restores enzyme stimulation (102). A study by Yuhanna et al. suggested that HDL can bind to endothelial SR-BI and thus directly stimulate eNOS-mediated NO production (9). HDL binding to SR-BI initiates tyrosine kinase Src-mediated activation of phosphoinositide (PI) 3-kinase, which in turn activates Akt and the MAP kinase/extracellular signal-regulated kinase pathway (98). Activation of endothelial Akt by HDL stimulates phosphorylation of eNOS at serine residue 1177 (Figure 2) (10, 98), which is known to be an important regulatory mechanism leading to eNOS activation (103).

Another mechanism has also been identified whereby HDL can maintain endothelial cell NO production and availability in mice fed a high-cholesterol diet (83). These authors suggested that HDL-induced ABCG1-mediated efflux of oxysterols from endothelial cells plays a role since 7-ketosterol, a dietary oxysterol, accumulated in endothelial cells of ABCG1- deficient mice on a western diet (83). Interestingly, incubation of human aortic endothelial cells with HDL prevented 7-ketosterol-induced production of reactive oxygen species and disruption of the active eNOS dimer. Furthermore, HDL-mediated cholesterol efflux via ABCG-1 reduced the inhibitory interaction of eNOS with caveolin-1 and thereby restored eNOS activity in cholesterol-loaded endothelial cells (104). These data suggest that the ability of HDL to preserve endothelial function in the presence of hypercholesterolemia may, at least in part, relate to an increased endothelial efflux of oxysterols.

Various components of HDL have been suggested to play a role in its endothelial NO-stimulating capacity. In cultured endothelial cells, the potential interaction of apoA-I with eNOS has been reported (99). However, despite being the ligand for SR-BI, a major HDL receptor, lipid-free apoA-I failed to activate eNOS, suggesting that other HDL components may be important or are required to support the conformation of apoA-1 to allow it to interact with SR-BI and to stimulate eNOS (105). In isolated endothelial cell plasma membranes, anti-apoA-I antibody inhibits eNOS activation by HDL, whereas anti-apoA-II antibody further enhances eNOS stimulation by HDL (9). Several studies have suggested that HDL-associated lysophospholipids may play a role in eNOS activation. Of note, HDL-associated sphingosylphosphorylcholine, sphingosine-1-phosphate, lysosulfatide may cause eNOS-dependent relaxation of precontracted aortic rings from mice via binding to the lysophospholipid receptor S1P3 expressed on endothelial cells (10). The vasodilatory response to HDL, however, was not completely inhibited in S1P3 deficient mice (10).

Recently, we observed that the HDL-associated antioxidant enzyme paraoxonase-1 (PON1) as an important determinant of the capacity of HDL to stimulate endothelial NO production and to exert NO-dependent endothelial-atheroprotective effects (15). Inhibition of PON1 in HDL from healthy subjects impaired the capacity of HDL to stimulate endothelial NO production and HDL isolated from PON1-deficient mice failed to stimulate NO production in mouse aortic endothelial cells (15). Furthermore, inhibition of eNOS-mediated NO production prevented the inhibitory effects of HDL from healthy subjects on nuclear factor κ B (NF- κ B) activity, vascular cell adhesion molecule (VCAM)-1 expression and endothelial monocyte adhesion, suggesting that the capacity of HDL to stimulate endothelial NO production is important for these endothelial anti-inflammatory effects of HDL (15).

Alterations of the effects of HDL from patients with cardiovascular disease on endothelial NO availability

We and others have recently shown that direct endothelial effects of HDL from patients with CAD or diabetes are markedly altered when compared to HDL from healthy subjects. In contrast to HDL from healthy subjects, HDL from patients with diabetes or chronic kidney disease failed to stimulate endothelial cell NO production and to promote endothelial repair in a carotid artery injury model in mice (13, 106). Moreover, HDL from patients with either stable CAD or an acute coronary syndrome, in contrast to HDL from age- and gender-matched healthy subjects, inhibited rather than stimulated endothelial cell NO production and lost the capacity to limit endothelial inflammatory activation as well as to promote endothelial repair *in vivo* (15).

Notably, we have observed that malondialdehyde (MDA) content is elevated in HDL from patients with coronary disease as compared to HDL from healthy subjects, which may limit endothelial NO production (15) (Figure 2). The antagonistic action of MDA was determined to be mediated by lectin-type oxidized LDL receptor 1 activation of protein kinase C- β II, which inhibits Akt-activating phosphorylation at Ser473 and eNOS-activating phosphorylation at Ser1177 (Figure 2). Because MDA formation is decreased by HDL-associated PON1 (107), PON1 activity was evaluated and was found to be markedly decreased in HDL from patients with coronary disease (15). Furthermore, PON1 inactivation in HDL from healthy subjects results in greater protein kinase C- β II activation in cultured endothelial cells, decreased activating eNOS-Ser1177 phosphorylation, and increased inactivating eNOS-Thr495 phosphorylation, resulting in attenuated NO production. Furthermore, HDL from PON1-deficient mice failed to stimulate endothelial cell NO production (15). These observations suggest that alterations of HDL-associated PON1 may have a major impact on endothelial effects of HDL.

An inverse relationship between PON1 serum activity and cardiovascular events has been reported (108, 109). A recent analysis of SNPs for PON1 identified in genome wide association studies did not reveal a

significant association between the lead SNPs for PON1, that was associated with mildly reduced paraoxonase activity, and the risk of cardiovascular events (110). A difficulty with respect to PON1 is that it is not known to what extent the paraoxonase and arylesterase activities of the enzyme represent biologically relevant functions. We and others have observed important post-translational modifications of the enzyme, which could lead to further alterations of biological properties of the enzyme (15, 111).

Effects of HDL on endothelial cell inflammatory activation: *Experimental studies and alterations of the effects of HDL from patients with cardiovascular disease*

Atherosclerosis is a chronic inflammatory disease. Endothelial adhesion and subsequent infiltration and accumulation of monocytes/macrophages and T lymphocytes into the arterial intima represent critical steps in initiation and progression of atherosclerotic lesions (46). HDL has been shown to inhibit the expression of monocyte chemoattractant protein (MCP)-1, an important pro-inflammatory chemokine in endothelial cells (91, 112). Studies have demonstrated that native HDL and reconstituted HDL containing apoA-I or the apoA-I Milano mutant inhibit the expression of leukocyte adhesion molecules in endothelial cells that are activated by pro-inflammatory stimuli (113, 114). Furthermore, HDL has been suggested to inhibit endothelial monocyte adhesion induced by oxLDL (115) or TNF- α (116) and monocyte transmigration in co-cultures of human aortic endothelial cells and smooth muscle cells stimulated with LDL (91).

The potential anti-inflammatory effects of HDL have also been demonstrated by several *in vivo* studies. Administration of reconstituted human HDL in apoE-deficient mice reduced VCAM-1 expression and decreased monocyte/macrophage infiltration following carotid artery cuff injury (117). Recently, it was shown that apoA-I gene transfer resulting in increased HDL cholesterol plasma levels inhibited diabetes-induced myocardial mRNA expression of VCAM-1 and ICAM-1 in mice with streptozotocin-induced diabetic cardiomyopathy (118). In contrast, in a study in apoE-deficient mice with transgenic overexpression of human apoA-I, endothelial VCAM-1 expression monocyte adherence were not reduced

in early atherosclerotic lesions at the aortic branch sites, despite reducing aortic atherosclerotic lesion formation (119). Hence, these observations support the concept that the anti-inflammatory capacity of HDL is heterogeneous, depending on the pathophysiological conditions, that is also consistent with findings of recent studies demonstrating that the inhibitory effects of HDL isolated from different human subjects on TNF- α stimulated endothelial VCAM-1 expression varied considerably (87, 120). In human studies, it has been suggested that administration of reconstituted HDL increased the anti-inflammatory capacity of HDL from patients with type-2 diabetes (121).

Several mechanisms have been suggested to explain the inhibitory effects of HDL on endothelial inflammatory activation (8). HDL can inhibit activation of the endothelial pro-inflammatory transcription factor NF- κ B (116, 122). Impaired endothelial NO bioavailability and increased endothelial superoxide production have been implicated in activation of NF- κ B (123). Furthermore, it was demonstrated that endothelial anti-inflammatory effects of HDL are mediated via SR-BI, PDZK1, PI3 kinase, eNOS, and S1P receptors (124). Recently, we have observed that inhibition of eNOS-mediated NO production reduced the inhibitory effects of HDL from healthy subjects on nuclear factor κ B (NF- κ B) activity, VCAM-1 expression and endothelial monocyte adhesion, suggesting that the capacity of HDL to stimulate endothelial NO production contributes to these endothelial anti-inflammatory effects of HDL (15).

It has been proposed that apoA-1, the major protein constituent of HDL, is able to recapitulate the anti-inflammatory capacity of HDL. In an *in vivo* study, infusion of apoA-I to rabbits subjected to acute vascular inflammation reduced neutrophil infiltration and endothelial cell inflammatory activation (125). Furthermore, administration of apoA-1 mimetic peptides, D-4F and L-4F has been shown to reduce vascular inflammation in type I diabetic rats and improved insulin sensitivity in obese mice (126, 127). Furthermore, lipid-free apoA-I and rHDL treatment reduced the expression of chemokines and chemokines receptors *in vivo* and *in vitro* via modulation of NF- κ B and peroxisome proliferator-activated receptor γ (128). One of the potential mechanisms for the anti-inflammatory effect of apoA-I is by mediating cellular cholesterol efflux through ABCA1, an ATP-binding transporter (129, 130). Interestingly, apoA-I has also been shown

to attenuate palmitate-induced NF- κ B activation by reducing toll-like receptor-4 recruitment into lipid rafts (131).

Besides apoA-I, the lipid component of HDL has also been proposed to be important for the anti-inflammatory effects of HDL. *In vitro* studies using discoidal reconstituted HDL containing apoA-I as the sole protein suggested that inhibitory effects of HDL on endothelial cell adhesion molecule expression are also, at least in part, dependent on HDL-associated phospholipid species (132). The inhibition of cytokine induced expression of VCAM-1 by reconstituted HDL varied substantially when different phosphatidylcholine species were compared, indicating that the lipid composition of HDL influences its anti-inflammatory capacity and might be an important determinant of HDL functionality (8, 132).

Alterations of effects of HDL from patients with coronary disease, diabetes or chronic kidney dysfunction on endothelial inflammatory activation

Early studies have demonstrated that the anti-inflammatory capacity of HDL is lost during acute phase responses in both humans and rabbits, as shown by the impaired ability of the isolated HDL to protect LDL from oxidation and inhibit the adhesion of monocytes to endothelial cells (87, 88). Furthermore, the capacity of HDL to inhibit LDL-induced monocyte chemotactic activity has also been shown to be impaired in CAD patients (89), which can be improved following simvastatin therapy (30). Recent studies have also described that the HDL capacity to inhibit the expression of adhesion molecules and monocyte chemotactic activity is lost in patients with CAD, diabetes, as well as end-stage renal disease (15, 93, 133).

Various mechanisms have been proposed to account for the impaired endothelial anti-inflammatory effects of HDL. The decrease in HDL apoA-I levels in inflammatory states has been related to decreased apoA-I synthesis in the liver, accelerated HDL catabolism, and apoA-I replacement in HDL particles by serum amyloid A (SAA) (134, 135). Upon induction of the acute phase, SAA is able to replace apoA-I in small, dense HDL, resulting in reduced plasma levels of apoA-I (136). In rabbits and mice, SAA can completely replace apoA-I in a subset of small, dense HDL particles, therefore functioning as a structural

apolipoprotein (137). Accordingly, SAA was recently found to be enriched in HDL from ESRD patients, which correlated with its reduced anti-inflammatory capacity to inhibit monocyte chemoattractant protein-1 formation in vascular smooth muscle cells (133).

In addition, certain amino acid residues in apoA-I, such as methionine, cysteine, tyrosine, and lysine residues are susceptible to oxidative modifications (138, 139). *In vitro* study has demonstrated that MPO-catalysed oxidative modification of HDL or apoA-I converts HDL into a pro-inflammatory particle which promotes NF- κ B activation and endothelial VCAM-1 expression (140). Furthermore, glycation of HDL and apoA-I, a process that is known to occur in diabetes *in vivo* (141), has also been proposed to impact on the anti-inflammatory capacity of HDL (142). In contrast to normal lipid-free ApoA-I, glycated lipid-free apoA-I infusion did not decrease adhesion molecule expression following vascular injury (142). Glycation of HDL has also been shown to impair the HDL capacity to inhibit oxLDL-induced monocyte adhesion to human aortic endothelial cells *in vitro* (143).

Effects of HDL on endothelial cell apoptotic pathways: *Experimental studies and alterations of effects of HDL from patients with coronary disease*

Endothelial dysfunction and injury contribute to the pathogenesis of atherosclerosis (96, 144, 145). Experimental studies have shown that atherosclerotic lesion-prone vascular regions are characterized by a high endothelial cell turn-over (146), which has been attributed to an increased rate of endothelial cell apoptosis. Endothelial cell apoptosis has also been suggested to contribute importantly to the pathophysiology of coronary disease (147, 148). The capacity of HDL to attenuate endothelial cell apoptosis may therefore represent an anti-atherogenic property of HDL (149-152).

HDL inhibits apoptosis of endothelial cells induced by both death-receptor-mediated and mitochondrial mediated apoptotic pathways. HDL may inhibit apoptosis triggered by various proatherogenic factors, such

as TNF- α , oxLDL and growth factor deprivation (149-151). Both HDL-associated proteins and lipids have been suggested to contribute to the anti-apoptotic capacity of HDL. ApoA-I has been shown to inhibit endothelial cell apoptosis induced by oxLDL, VLDL, and TNF- α (150, 151, 153). HDL subpopulations enriched with apoA-I account for approximately 70% of the anti-apoptotic activity of HDL in human microvascular endothelial cells that were treated with mildly oxLDL and reconstitution of HDL with apoA-I, cholesterol and phospholipids potently decreased oxLDL-induced apoptosis in these cells (152), suggesting that apoA-I plays an important role for the anti-apoptotic capacity of HDL in oxLDL-stimulated endothelial cells.

HDL-associated lysosphingolipids have also been shown to inhibit endothelial cell apoptosis triggered by growth factor deprivation (149, 154, 155). The anti-apoptotic capacity of HDL-associated lipids was further supported by the findings that the ratio of sphingosine-1-phosphate and sphingomyelin was increased in small dense HDL3 particles and positively correlated with the capacity of these HDL subpopulations to attenuate endothelial cell apoptosis (156).

Several mechanisms have been proposed for the endothelial anti-apoptotic effects of HDL, depending on the trigger of apoptosis. OxLDL causes a delayed but sustained increase in intracellular calcium in endothelial cells, leading to cell death, and this effect is reversed by HDL and mediated by prevention of the calcium increase (151). Tumor necrosis factor- α -induced endothelial cell apoptosis is also inhibited by HDL, and this is associated with attenuated induction of CPP32-like protease (caspase 3), which is a component of all primary apoptotic pathways (150). Growth factor deprivation activates the mitochondrial pathway of apoptosis, which can be suppressed by HDL. HDL inhibits the dissipation of mitochondrial potential, oxygen-derived free radical generation, cytochrome c release to the cytoplasm, and activation of caspase 3 and caspase 9. HDL also activates Akt and causes phosphorylation of the Akt target Bcl-2-associated death promoter Bad, preventing it from binding to Bcl-xL (Figure 2). Bcl-xL, an anti-apoptotic Bcl-2 family protein, is then free to inhibit mitochondria-mediated apoptosis (149). In addition, HDL causes phosphoinositide 3 (PI3) kinase mediated up-regulation of the Bcl-xL expression (16). Interestingly, HDL

retained its anti-apoptotic activity after knockdown of eNOS using specific RNA interference or pharmacological inhibition using L-NAME (16), suggesting that HDL may exert its anti-apoptotic activity independently of eNOS activation. The lysophospholipid sphingosine-1-phosphate (S1P) enhances endothelial cell survival, and these effects are inhibited by knockdown of the S1P receptor endothelial differentiation gene-1/S1P1 by pertussis toxin and by phosphoinositide 3 (PI3) kinase and Erk pathway antagonists, suggesting that signaling by lysophospholipid components of HDL may be important for the inhibition of apoptosis (155).

Alterations of the effects of HDL from patients with cardiovascular disease on endothelial apoptosis

Recently, we have observed that reduced clusterin and increased apolipoprotein C-III content in HDL isolated from patients with CAD lead to activation of pro-apoptotic signaling pathways in endothelial cells (16). In contrast to HDL from healthy subjects, HDL isolated from patients with stable CAD or an acute coronary syndrome failed to inhibit endothelial cell apoptosis *in vitro* and in apoE-deficient-mice *in vivo*. Instead, HDL isolated from these patients stimulated endothelial pro-apoptotic pathways, in particular p38-MAPK-mediated activation of the pro-apoptotic Bcl-2-protein tBid (Figure 2). Our studies further suggest that differences in the proteome of HDL from patients with CAD, in particular reduced HDL-associated clusterin and increased HDL-associated apoC-III, play an important role for altered activation of endothelial anti- and pro-apoptotic signaling pathways (Figure 2) (16). Furthermore, oxidative modifications of HDL may also play a role in the loss of anti-apoptotic activity of HDL, as demonstrated by Undurti et al. showing that MPO-catalyzed oxidation of HDL resulted in the impaired capacity to inhibit endothelial apoptosis *in vitro* (140).

Effects of HDL on endothelial repair after vascular injury: *Experimental studies and effects of HDL from patients with cardiovascular disease*

Recent studies have also suggested that HDL may stimulate endothelial repair processes. Endothelial repair processes have long been thought of to be only dependent on the proliferation and migration of local adjacent endothelial cells (146), however several recent studies have demonstrated that bone-marrow derived mononuclear cells (early outgrowth cells; EOCs) can promote endothelial repair after vascular injury (157, 158), and likely contribute to endothelial repair processes in lesion-prone areas of experimental atherosclerosis and improve endothelial function (159, 160).

HDL stimulates endothelial repair by promotion of endothelial cell proliferation or migration and stimulation of the recruitment and endothelial repair capacity of EOCs (13, 161, 162). HDL induces a marked increase in endothelial cell migration *in vitro* with effects comparable to endothelial growth factors, such as basic fibroblast growth factor or vascular endothelial growth factor (155, 161, 163). Native HDL and the HDL-associated lysosphingolipid sphingosine-1-phosphate stimulate endothelial cell migration via sphingosine-1-phosphate receptors S1P1 and S1P3 and the effects could be blocked by pertussis toxin which inhibits the interactions between G proteins and G protein-coupled receptors (155). The importance of sphingosine-1-phosphate for endothelial cell migration was supported by another study demonstrating that sphingosine-1-phosphate induced tube formation of human coronary artery endothelial cells *in vitro* by Ras/Raf1-dependent ERK activation (164). In contrast, in a work by Seetharam et al. pertussis toxin did not affect HDL-mediated endothelial cell migration (161), suggesting the presence of another pathway and agonist, which induces the migration of endothelial cells by HDL. Indeed, the authors observed that reconstituted HDL consisting of apoA-I, palmitoylcholine and cholesterol was able to induce endothelial cell migration (161). Moreover, native HDL induced rapid changes in the actin cytoskeleton of endothelial cells (i.e. a decrease in stress fibers, an increase in lamellipodia, and membrane ruffling) paralleled by an activation of the small GTPase Rac, that is known to mediate lamellipodia formation (161). Interestingly, the authors were able to demonstrate that endothelial Rac activation and

migration in response to HDL is independent of endothelial NO production, but requires binding of HDL to SR-BI and activation of Src kinase, PI3-kinase and MAP kinase (161). PDZ domain-containing protein PDZK1 was identified as an adaptor protein of SR-BI in endothelial cells and it was suggested that PDZK1 is required for the initiation of HDL signalling *via* SR-BI in endothelial cells and plays an important role for endothelial cell migration induced by HDL (162). Interestingly, further studies have suggested that HDL and SR-BI promote re-endothelialization of the carotid artery after perivascular electric injury in mice (13, 161). In this model, carotid artery re-endothelialization was impaired in apoA-I deficient mice with low HDL levels as well as in SR-BI deficient mice. Of note, reconstitution of apoA-I expression by liver-directed apoA-I gene transfer with subsequent normalisation of HDL plasma levels restored the re-endothelialization response in apoA-I deficient mice, strongly suggesting that apoA-I and HDL promote endothelial monolayer integrity *in vivo* (161). In another study, elevation of HDL levels in apoE-deficient mice induced by adenoviral human apoA-I (AdA-I) transfer increased the number of Flk1 / Sca-1 double-positive cells in peripheral blood and the number of DiIacLDL / FITC-isolectin double positive cells after 4 days of *ex vivo* culture of spleen mononuclear cells (165).

Besides increasing the number of EOCs, AdA-I transfer in apoE-deficient mice improved the migratory capacity of spleen-derived early EOCs in response to HDL, the adhesion of spleen-derived early EOCs to fibronectin and the invasion of spleen-derived early EOCs in solidified Matrigel (165). Finally, AdA-I transfer also promoted the incorporation of EOCs in Balb/c common carotid artery allografts transplanted paratopically in C57BL/6 ApoE^{-/-} mice that was associated with an increase in endothelial regeneration and inhibition of transplant arteriosclerosis (165). In a follow-up study, the same group observed that murine and human early EOC express SR-BI, as indicated by immunocytochemistry analysis of human early EOCs and murine spleen-derived early EOCs after 4 and 7 days of *ex vivo* culture (166). Of note, the authors did not observe an increase in circulating Flk1 / Sca-1 double-positive cells and DiI-acLDL / FITC-isolectin double positive cells after *ex vivo* culture of spleen mononuclear cells after AdA-I transfer in mice transplanted with SR-BI deficient bone marrow, suggesting that expression of SR-BI in bone marrow is

critical for EOC mobilisation induced by HDL (166). Furthermore, the migratory capacity of bone-marrow derived early EOC deficient in SR-BI in response to HDL was reduced as compared to early EOCs containing SR-BI and this was at least in part due to an impaired activation of extracellular signal-regulated kinases (ERK) and decreased NO production in SR-BI deficient early EOCs (166). *In vivo*, SR-BI deficiency in bone marrow abrogated the inhibitory effect of AdA-I transfer on allograft vasculopathy after paratopical transplantation of a common carotid artery of a female BALB/c donor mouse into the recipient male C57BL/6 mice, that was paralleled by impaired endothelial regeneration and EOC incorporation in allografts (166). Besides increasing HDL levels by adenoviral human apoA-I transfer, intravenous infusion of reconstituted HDL has also been shown to increase the number of Sca-1 positive cells in the aortic endothelium of apoE deficient mice, supporting a role for HDL in promoting endothelial repair (167). Furthermore, intravenous injection of reconstituted HDL increased blood flow recovery and capillary density in a murine ischemic hindlimb model (168).

Of note, a pivotal role for eNOS in the regulation of EOC mobilization was shown using eNOS-deficient mice, which demonstrated an impaired capacity to mobilize EOCs and impaired function of isolated EOCs (169). Studies have demonstrated that colony-forming capacity and migratory function of circulating EOCs are impaired in conditions associated with reduced NO bioavailability, suggesting a link between eNOS activity and EOC function (170, 171). The effects of HDL on circulating EOCs may also involve increasing cell survival and prevention of apoptosis. HDL prevents apoptosis of early EOCs through inhibition of caspase-3 activity (172). Furthermore, administration of D-4F has been shown to cause EOCs to produce a robust increase in eNOS and heme oxygenase-1 thereby enhancing its survival which may contribute to vascular repair in diabetic rats (126).

Alterations of the effects of HDL from patients with diabetes or CAD on endothelial repair processes

Reconstituted HDL has been described to improve EOC availability in patients with type 2 diabetes, which have a reduced availability and impaired function of EOCs (173). HDL from patients with type 2 diabetes

has recently been found to have a diminished capacity to stimulate endothelial cell proliferation, migration and adhesion to extracellular matrix and this impairment is associated with down regulation of SR-BI expression (174). Notably, we have observed that HDL from patients with CAD has impaired endothelial repair capacity following vascular injury *in vivo* using nude mice carotid artery injury model (15).

Effects of HDL on endothelial thrombotic activation: *Experimental studies and alterations of the effects of HDL from patients with diabetes*

There has been some direct evidence demonstrating potential anti-thrombotic actions of HDL. In a rat model of acute arterial thrombosis, infusion of apoA-I Milano caused a prolongation in the time of thrombus formation and a reduction in the weight of the thrombus (175). In humans, infusion of reconstituted HDL limited development of a procoagulant state in healthy volunteers given low doses of endotoxin (176). One potential mechanism contributing to anti-thrombotic effects of HDL is an increased endothelial prostacyclin synthesis. Prostacyclin acts synergistically with NO to induce vascular smooth muscle relaxation, inhibits platelet activation, and diminishes the release of growth factors that stimulate the local proliferation of smooth muscle cells (177). Incubation with native HDL increases prostacyclin production in cultured endothelial cells (178, 179), and the effects can be recapitulated partially by delipidated HDL (180), suggesting that both HDL-associated lipids and apolipoproteins are involved in the process. Prostacyclin release has also been shown to increase when isolated rabbit and rat hearts are infused with HDL (181, 182). The impact of HDL on prostacyclin production in endothelium occurs by both the provision of arachidonate (178, 180, 183) and upregulation of Cox-2 expression (184, 185). It has also been observed that HDL3 induces Cox-2 expression and prostacyclin release via a p38 MAP kinase/CREB-dependent pathway in the endothelium (185-188). Recently, it was demonstrated that apoA-I, but not apoA-II, induced the expression of Cox-2 and the production of prostacyclin through the p38 MAPK, ERK1/2 and JAK2

pathways via ABCA1 in endothelial cells (189). Involvement of SR-BI mediated PI3K-Akt-eNOS signaling in HDL-induced Cox-2 expression and prostacyclin release in endothelial cells has also been reported (190).

Increased tissue factor and selectin expression on platelets and endothelial cells have been identified as critical factors in the initiation of thrombus formation. The phospholipid components of HDL have been demonstrated to contribute to the downregulation of E-selectin expression on endothelial cell surfaces (8, 184). Furthermore, reconstituted HDL has been shown to downregulate thrombin-induced endothelial cell tissue factor expression *in vitro* (191). HDL has also been shown to exert antithrombotic effects by inhibiting platelet activation. The administration of reconstituted HDL to humans or the infusion of apoA-I Milano into rats inhibits platelet aggregation (176, 192). HDL may regulate platelet function by downregulating the release of platelet activating factor or by upregulating endothelial NO synthesis and release (185, 193). HDL also downregulates the biosynthesis of thromboxaneA2 (TxA2) and upregulates prostacyclin production which can decrease platelet aggregation as well as blunt leukocyte-endothelial cell interactions and thereby prevent the initiation and progression of atherogenesis (194).

Alterations of the effects of HDL from patients with diabetes on endothelial thrombotic activation

While there is no direct evidence showing that the anti-thrombotic actions of HDL may be impaired in inflammatory conditions, the involvement of PI3K-Akt-eNOS pathways (190) and also p38 MAPK, ERK1/2 and JAK2 pathways (185, 189) in HDL-induced Cox-2 expression and prostacyclin release suggests that disruption in these pathways may likely leads to altered actions of HDL on endothelial cells relevant to thrombosis.

However, interestingly, a study showed that oxidized HDL strongly inhibits platelet activation and aggregation (195). This anti-thrombotic action of oxidized HDL is mediated by SR-BI but independently of eNOS/Akt pathway (195). More recently, HDL from patients with early stages of type 2 diabetes has been reported to upregulate Cox-2 expression and prostacyclin release in endothelial cells, mediated via S1P receptors (196). The authors suggest that the increased in HDL-associated S1P levels in the early stage

of type 2 diabetes may contribute to the anti-thrombotic actions of HDL indicating a compensatory protective mechanism during the early course of the disease (196). More studies will be needed in this area to better understand the interaction between HDL and thrombotic pathways.

Conclusion and Perspectives

Over the past two decades numerous studies have revealed several direct effects of HDL on endothelial cell functions, including potentially anti-atherogenic properties. It has become clear, that HDL particles are highly heterogeneous and the vasoprotective effects of HDL are altered in patients with coronary disease, diabetes and chronic kidney dysfunction, i.e. patients with a high cardiovascular risk profile. Most of the studies examining functions of HDL from healthy subjects or patients with cardiovascular disease have been performed *ex vivo* i.e. by using isolated HDL from different study populations and testing it in *in vitro* bioassays. While these studies provide clear evidence for the heterogeneity of HDL particles and its altered effects on vascular cells, when HDL is obtained from patients with cardiovascular disease as compared to healthy subjects, the *in vivo* relevance of these functional alterations of HDL and the potential role for progression of coronary disease remains to be determined. Notably, however, these findings have more recently been paralleled by observations in cohort studies, suggesting that in patients with advanced coronary disease, higher plasma levels of HDL cholesterol are no longer associated with a reduced risk of cardiovascular events (23, 197).

Of note, the functional heterogeneity of HDL may be attributed to the complexity of the HDL particles, containing more than 50 proteins and numerous lipids that can be modified or altered in their composition. Recent advances in the field of HDL proteomics and lipidomics have been reviewed by Shah et al. (198) and Kontush et al. (199), respectively, in the same thematic review series by the *Journal of Lipid Research*. We are just beginning to understand which HDL subspecies may be responsible for specific vasoprotective properties of HDL. It is noteworthy that rapid advances in the MS proteome technology have allowed

tremendous leaps in characterizing the HDL proteome. Studies investigating the lipidome of HDL are still somewhat limited by the available technologies.

Taken together, plasma HDL cholesterol levels are likely not an appropriate marker of vascular effects of high-density lipoproteins, and therefore itself do not represent a reliable therapeutic target. HDL-targeted treatment approaches likely need to take into account the altered vascular effects of HDL in cardiovascular disease. Moreover, targeting HDL-mediated anti-atherogenic mechanisms, rather than HDL cholesterol plasma levels, may represent a promising and interesting therapeutic target. Further studies into the specific mechanisms leading to a loss of anti-atherogenic effects of HDL are in particular warranted, in order to determine which therapeutic approaches may be particularly potent in restoring HDL's vasoprotective properties. The effect of such therapeutic approaches on HDL function will have to be tested in future studies.

Acknowledgements

This work was supported by the Leducq Foundation, the Swiss National Science Foundation (SNF-138486), the Swiss Heart Foundation, and the Zurich Integrative Human Physiology (ZIHP) grant. Conflict of interest statement: U.L. has received speaker fees and a research grant from Roche and Merck. M.R. has no conflict of interest. We apologize for not citing all worthy studies due to space limitations.

Figure Legends

Figure 1. *Left panel* – HDL from healthy subjects exerts direct vasoprotective effects, such as endothelial anti-inflammatory, anti-oxidative, anti-apoptotic and anti-thrombotic effects. HDL from healthy subjects stimulates endothelial cell nitric oxide production and promotes endothelial repair after vascular injury. *Right panel* - Accumulating evidence suggests that the vascular effects of HDL can be highly heterogeneous. HDL loses potential anti-atherosclerotic properties in patients with chronic inflammatory diseases, such as coronary artery disease, that has been termed “HDL dysfunction”. Of note, HDL may also become a proinflammatory particle in certain pathophysiological conditions.

Figure 2. Signaling pathways mediating the effects of HDL on endothelial NO production and endothelial apoptosis. HDL from healthy subjects binds to SR-BI via apoA-I, leading to PDZK1-dependent activation of Src family kinases, PI3K and Akt, which phosphorylates eNOS at serine residue 1177, therefore increasing eNOS activity. PI3K-dependent MAPK activation and binding of HDL-associated lysophospholipids to the S1P3 receptor also activate eNOS. In contrast, HDL from patients with CAD can suppress eNOS activation. Inactivation of PON1 and greater accumulation of MDA in HDL lead to LOX-1-mediated activation of PKC β II and inhibition of eNOS by phosphorylation at threonine 495. HDL-associated clusterin promotes endothelial anti-apoptotic signaling via activation of PI3K and Akt leading to increased expression of anti-apoptotic Bcl-xL. Akt activation also phosphorylates Bcl-2-associated death promoter Bad, preventing it from binding to Bcl-xL, which is then free to inhibit mitochondria-mediated apoptosis. In CAD, the level of HDL-associated clusterin is reduced, whereas HDL-associated apoC-III content is increased. HDL-associated apoC-III activates MAPK signaling via phosphorylation of p38 leading to increased activation of pro-apoptotic tBid, which promotes cytochrome C release from the mitochondria and downstream caspase-3 mediated apoptosis.

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Table 1. Summary of the endothelial vasoprotective effects of High-density lipoprotein and its alterations in pathophysiological states

Endothelial function	Mechanisms of effects of HDL	Key components	Alterations in Pathophysiological Settings
Endothelial Protection by limiting LDL oxidation	HDL prevents oxidation of LDL <i>in vitro</i> [41-43]	ApoA-I, ApoE, ApoJ, PON1, LCAT, PAF-AH	Impaired antioxidant capacity of HDL in patients with CAD [89, 90, 94] and ischaemic cardiomyopathy [95]
	HDL is a major carrier of lipid peroxidation products [44,45]		Loss of antioxidant capacity of HDL after surgery in humans and rabbits [87] and after acute influenza in mice [88]
Stimulation of Endothelial NO production	Direct stimulation of eNOS via SR-BI and S1P3 [9,10,13,15,97-99]	ApoA-I, sphingosine-1-phosphate, PON1	Impaired capacity of HDL to stimulate endothelial NO production in patients with CAD [15], diabetes [13], CKD[106], antiphospholipid syndrome [25]
	Reduce inhibitory interaction of eNOS with caveolin-1 via ABCG1-mediated oxysterol efflux [83,104]		
	Prevention of oxLDL-mediated eNOS displacement from caveolae [102]		
Impact on Endothelial Inflammatory Activation	Inhibition of endothelial monocyte adhesion <i>in vitro</i> [91,112-116]	ApoA-I, phosphatidylcholine	Impaired anti-inflammatory capacity of HDL in patients with CAD [15], diabetes [93] and ESRD [133]
	Inhibition of endothelial VCAM-1 and ICAM-1 expression <i>in vitro</i> and <i>in vivo</i> [117-121]		Displacement of apoA-I by SAA in rabbits and mice [134-137]
			Impaired anti-inflammatory capacity of apoA-I after MPO-mediated oxidation <i>in vitro</i> [140], and of HDL after glycation <i>in vitro</i> [142,143]
Impact of Endothelial Apoptotic Pathways	Inhibition of endothelial apoptosis mediated by TNF- α , oxLDL, growth factor deprivation <i>in vitro</i> [149-151]	ApoA-I, lysosphingolipids, clusterin	Impaired anti-apoptotic capacity of HDL in patients with CAD with reduced clusterin and increased apoC-III content [16]
			Reduced anti-apoptotic capacity of HDL after MPO-catalyzed oxidation <i>in vitro</i> [140]
Impact on Endothelial Repair after vascular injury	Stimulation of endothelial cell proliferation, migration and recruitment of early EOC <i>in vitro</i> and <i>in vivo</i> [13,161,162]	ApoA-I, sphingosine-1-phosphate	Impaired HDL capacity to stimulate endothelial repair after vascular injury in patients with type 2 diabetes [173,174] and CAD [15]
	Prevention of apoptosis of circulating EOCs [172]		
Impact on Endothelial Thrombotic Activation	Increased prostacyclin synthesis <i>in vitro</i> [178,179] and <i>in vivo</i> [181,182]	ApoA-I, phospholipids	Oxidized HDL strongly inhibits platelet activation and aggregation [195]
	Stimulation of Cox-2 expression [185-188] and inhibition of E-selectin expression [8,184]		HDL isolated from patients with type 2 diabetes upregulates Cox-2 expression and prostacyclin release [196]
	Downregulation of endothelial cell tissue factor expression <i>in vitro</i> [191]		

Figure 1

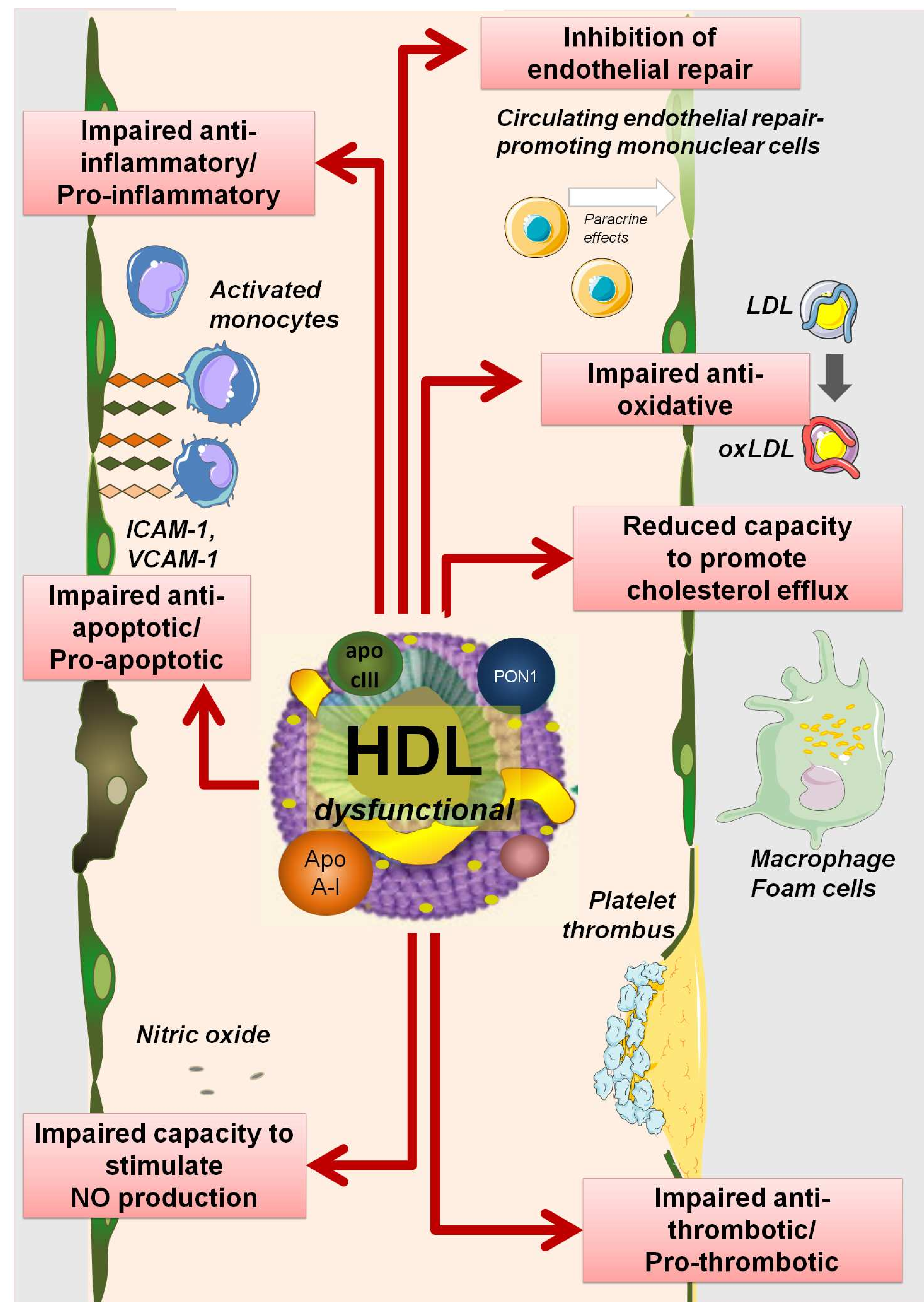
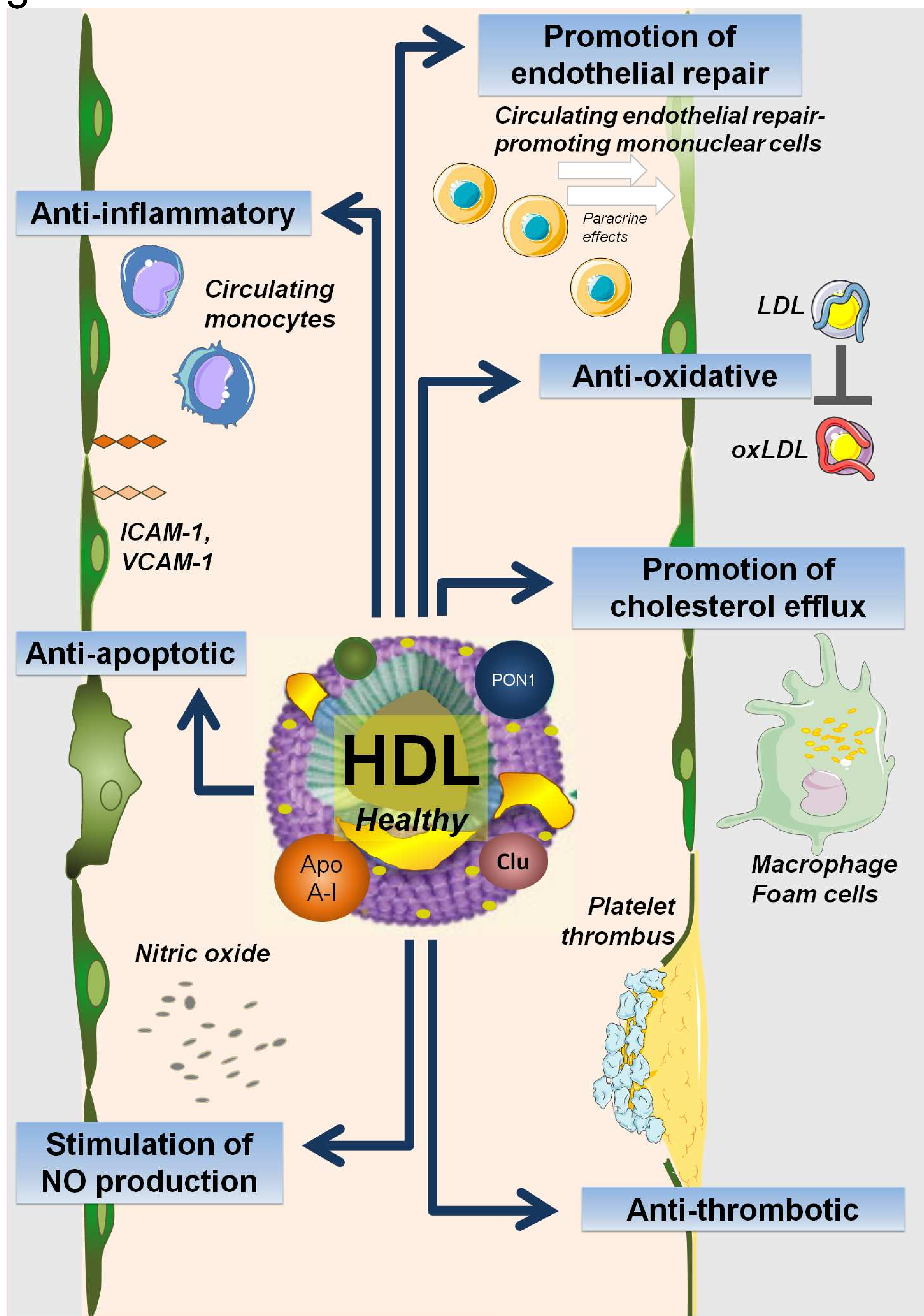


Figure 2

